

(2-16-95)

MRID No. 428990-05

DATA EVALUATION RECORD

1. **CHEMICAL:** Oxine Copper.
Shaughnessey No. 024002.
2. **TEST MATERIAL:** 1) Quinolinate 98; oxine copper or copper 8-quinolinolate; Batch No. 52390; 100% active ingredient; a green powder. 2) ^{14}C -oxine copper; Lot No. 041H9267; specific activity of 63.78 $\mu\text{Ci}/\text{mg}$; 98% active ingredient.
3. **STUDY TYPE:** 72-4. Freshwater Invertebrate Life-Cycle Test.
Species Tested: *Daphnia magna*.
4. **CITATION:** Ward, G.S. 1993. Oxine Copper (Copper 8-Quinolinolate): Chronic Toxicity to the Water Flea, *Daphnia magna*, Under Flow-Through Test Conditions. Laboratory Project ID No. J9006014f. Study conducted by Toxikon Environmental Sciences, Jupiter, FL. Submitted by LA QUINOLEINE et ses dérivés, S.A., Paris, France. EPA MRID No. 428990-05.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: 

Date:  2/16/95

6. **APPROVED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: 

Date: 12/13/93

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: 

Date: 2/16/95

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a chronic toxicity study. Dissolved oxygen concentrations fell to extremely low (16% of saturation) levels during the test period and the highest measured concentration in one exposure solution was greater than twice the lowest measured concentration at the same level. Under the conditions of the test, the MATC, based on the most sensitive biological parameter (length), was $>6.4 \mu\text{g ai}/\text{l}$ and $<10.4 \mu\text{g ai}/\text{l}$, based on mean measured concentrations. The geometric mean MATC was $8.2 \mu\text{g ai}/\text{l}$.

8. RECOMMENDATIONS: N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: *Daphnia magna* (<24 hours old) were obtained from in-house cultures which were originally received from U.S. Environmental Protection Agency, Duluth, Minnesota. The cultures were maintained in moderately-hard dilution water at a temperature of $20 \pm 2^\circ\text{C}$.

B. Test System: The flow-through test system was a modified proportional (50%) vacuum-siphon diluter. A test solution volume of 250 ml was delivered to each test tank during every cycle; the total volume was split into fourths (approximately 62 ml) via a splitter box prior to entry into the test chambers. Test tanks were 11.5-l glass aquaria equipped with automatic glass siphons. The siphons were positioned to provide a maximum depth of 6 cm. Within each test tank, four replicate test chambers were positioned to receive incoming flow of test solution from the splitter box. The test chambers were 100- x 50-mm glass crystallizing dishes with a band of 315- μm mesh screening attached with silicone sealant to the rim of the dish. Each test chamber maintained a 300-ml volume of test solution. The diluter cycled at an average rate of 2.5 cycles per hour providing approximately 12.5 volume additions every 24 hours.

A photoperiod of sixteen hours of light per day at an intensity of 278-385 lux with 15 minute transition periods of lower light intensity was used throughout the study. Test tanks were randomly positioned in a temperature-controlled water bath.

The dilution water, which was carbon-treated Jupiter, Florida, town water, was vigorously aerated prior to use. The total hardness and alkalinity of the dilution water were 36-72 and 20-28 mg/l as CaCO_3 , respectively. The specific conductivity of the test water was 382-480 $\mu\text{mhos/cm}$.

A stock solution containing 1.6% radiolabeled material and 98.4% unlabeled material was prepared in acidified dimethylformamide (DMF). The concentration of oxine

copper in the stock solution was 1 mg active ingredient (ai)/ml. The stock solution was pumped into the diluter mixing chamber providing the highest nominal test concentration of 100 $\mu\text{g ai/l}$. The mixing chamber solution was proportionally diluted to provide the lower-concentration treatment solutions.

- C. **Dosage:** Twenty-one-day, flow-through test. Nominal test concentrations selected based on results of a range-finding study were 3.13, 6.25, 12.5, 25, 50, and 100 $\mu\text{g ai/l}$. A solvent and dilution water control were also prepared. The solvent control and all exposure solutions had a solvent concentration of 0.1 ml DMF/l.
- D. **Design:** Ten daphnid neonates were impartially assigned (by twos) to each test chamber, four test chambers per vessel, for a total of 40 daphnids per treatment or control. The screen chambers were randomly placed in the test vessels.

An algal suspension (*Selenastrum capricornutum*) of 1.48×10^5 cells/ml was maintained in the test solutions. In addition, 9.4 mg of a yeast/trout chow/cerophyll suspension was added daily to each test chamber.

Survival and reproduction were monitored daily. Dead daphnids were removed. All young were counted and discarded. At test termination (day 21 of exposure), the length of all surviving daphnids was determined to the nearest 0.1 mm.

Test solutions were not aerated during the study. Dissolved oxygen concentration (DO), specific conductivity, and pH were measured on days 0, 7, 14, and 21. Temperature was monitored hourly. The hardness and alkalinity of the control and the low, middle, and high treatment concentration solutions were measured weekly.

Water samples were collected from each treatment and control solution on test days 0, 3, 5, 7, 10, 14, and 21 to monitor actual exposure concentrations. A sample from each replicate chamber was taken and the four samples were pooled. The concentration of oxine copper in the solutions was determined using liquid scintillation counting.

- E. **Statistics:** The EC_{50} values were calculated by a computer program which employed the moving average

angle, probit, logit, and non-linear interpolation methods.

The dilution water control and solvent control were statistically compared. If a significant difference was determined, the solvent control was used for comparison to the treatments. Control data were pooled if no significant differences were detected.

Mortality data were analyzed using analysis of variance (ANOVA) and Dunnett's procedure ($p \leq 0.05$). An arcsine square root transformation was applied to the mortality data. Reproduction data (young/adult reproduction day) were analyzed using a one-way ANOVA following normalization. The length data were analyzed using a one-way analysis of variance (ANOVA) without transformation or normalization. The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean between the most sensitive no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC).

12. **REPORTED RESULTS:** Mean measured concentrations were 2.35, 6.40, 10.4, 18.2, 35.1, and 70.9 $\mu\text{g ai/l}$ which ranged from 70 to 102% of nominal values (Table 1, attached).

After 21 days of exposure to oxine copper, daphnid mortality in the dilution water control (10%) was not statistically different from that in the solvent control (12%). Daphnid survival at the highest treatment concentration of 70.9 $\mu\text{g ai/l}$ was significantly reduced when compared to the pooled control (Table 2, attached). Both the 14- and 21-day EC_{50} was 63.9 $\mu\text{g ai/l}$ (95% confidence interval = 35.1 $\mu\text{g ai/l}$ to infinity).

Eggs were first observed on day 4 in the control and all treatment chambers. The average number of young produced per adult reproductive day in the dilution water control group (16.7) was statistically different from that in the solvent control group (10.0). The average number of young produced per adult reproductive day was significantly reduced at the two highest treatment levels in comparison to the solvent control (Table 4, attached).

Growth (length) of the dilution water and solvent control daphnids was also statistically different. Length was significantly reduced at the 10.4 and 18.2 $\mu\text{g ai/l}$ levels when compared to the solvent control (Table 5, attached).

During the study, the temperature ranged from 17.8 to 22.8°C, the pH ranged from 6.9 to 8.2, the specific conductivity ranged from 382 to 614 $\mu\text{mhos/cm}$; and the DO ranged from 1.5 to 9.1 mg/l (16 to 100% of saturation). The hardness and alkalinity ranges of the dilution water were 36-72 and 20-28 mg/l as CaCO_3 , respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The NOEC was 6.4 $\mu\text{g ai/l}$ based on the lack of significant mortality, reproductive, or growth effects at this concentration. The LOEC was 10.4 $\mu\text{g ai/l}$ based upon a significant reduction in growth. Therefore, the MATC for oxine copper was >6.4 but <10.4 $\mu\text{g ai/l}$.

A Good Laboratory Practice (GLP) compliance statement was included in the report indicating that the study was conducted in accordance with EPA GLP standards. A Quality Assurance statement was also included in the report.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

- A. **Test Procedure:** An SEP for *Daphnia magna* chronic flow-through studies is not available at this time, thus the SEP for the *Daphnia magna* life-cycle (21-day renewal) chronic toxicity test was used as a general guide in the validation process. Study weaknesses are noted as follows:

The history of the parents of the test daphnids was not reported. The parents should have been held for at least 21 days in dilution water at test temperature and have had at least one brood before collection of the test daphnids.

During the test, the DO in some test solutions was as low as 16% of saturation (Table 8, attached). Raw water quality data were not reported; therefore, the reviewer cannot determine whether the DO remained $<50\%$ of saturation for more than 48 hours, a condition which is considered unacceptable.

The hardness, alkalinity, and conductivity should have been measured in at least one exposure solution.

The author did not report the method by which the concentration of solvent in the exposure solutions was made the same as that in the solvent control.

At the nominal concentration of 6.25 $\mu\text{g ai/l}$, the highest measured concentration was more than twice the lowest measured concentration.

Daphnid length should have been measured to the nearest 0.01 mm, rather than the nearest 0.1 mm.

Daphnid weight was not measured. EPA prefers weight to length data since the weight data are more reliable.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal computer program to determine the 21-day LC_{50} and obtained similar results (printout, attached). Williams' test was used to analyze survival and reproduction data in comparison to the solvent control data (printouts, attached). Survival data were arcsine square-root transformed before analysis. Two-way ANOVA and Bonferroni's test were used to analyze the length data in comparison to the solvent control data (printouts, attached). The results for reproduction were the same as the author's, but no significant differences were observed for the length analysis. Since the author's NOEC and LOEC were more conservative than the reviewer's, they will be reported and used to determine the MATC.

C. **Discussion/Results:** This study is not scientifically sound and does not meet the guideline requirements for a chronic toxicity study. The MATC, based on the most sensitive biological parameter (length), was $>6.4 \mu\text{g ai/l}$ and $<10.4 \mu\text{g ai/l}$, based on mean measured concentrations. The geometric mean MATC was $8.2 \mu\text{g ai/l}$.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** Dissolved oxygen concentrations fell to extremely low (16% of saturation) levels during the test period and the highest measured concentration in one exposure solution was greater than twice the lowest measured concentration at the same level.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 11-19-93.

Table 1. Measured Concentrations of Oxine Copper During a 21-Day Exposure of Water Flea, Daphnia magna, Under Flow-Through Conditions

Nominal Concentration ($\mu\text{g/L}$; ppb)	Measured Concentration ($\mu\text{g/L}$; ppb)										Percent of Nominal
	0 D	3 D	5 D	7 D*	10 D	14 D	21 D	Mean ($\pm\text{SD}$)			
Control	<0.93	<0.93	<0.93	<0.93	<0.93	<0.93	<0.93	<0.93	<0.93	<0.93	---
Solvent Control	<0.93	<0.93	<0.93	<0.93	<0.93	<0.93	<0.93	<0.93	<0.93	<0.93	---
3.13	2.60	2.77	2.82	2.35	1.94	1.93	2.05	2.35(0.42)		75	
6.25	6.06	11.1	6.00	8.21	5.58	4.38	5.30	6.40(2.38)		102	
12.5	8.28	13.0	11.6	11.9	11.3	8.73	9.27	10.4 (1.87)		83	
25.0	19.8	17.9	19.5	20.4	17.4	15.9	18.4	18.2 (1.43)		73	
50.0	39.8	32.1	37.7	33.6	36.7	30.2	34.1	35.1 (3.62)		70	
100	75.5	68.1	80.4	74.3	68.1	63.2	70.0	70.9 (6.12)		71	
----- SPIKE RECOVERY DATA (As Percentages) -----											
MS	A	87	82	76	71	90	94	99			---
	B	81	83	82	75	96	93	96	88 (± 7)		---

MS = Matrix spike.

* Matrix (QC) spike sample below acceptability. Concentrations not utilized in calculation of means.

Table 2. Mortality of Water Flea, *Daphnia magna*, Exposed to Oxine Copper Under Flow-Through Test Conditions

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Rep	Cumulative Number Dead (% Mortality)				Treatment ^a
		Day 3	Day 7	Day 14	Day 21	
Control	A	1 (10)	1 (10)	1 (10)	1 (10)	
	B	1 (10)	1 (10)	1 (10)	2 (20)	
	C	0 (0)	0 (0)	0 (0)	1 (10)	
	D	0 (0)	0 (0)	0 (0)	0 (0)	4 (10)
Solvent Control	A	2 (20)	2 (20)	2 (20)	2 (20)	
	B	1 (10)	1 (10)	1 (10)	1 (10)	
	C	0 (0)	0 (0)	0 (0)	1 (10)	
	D	1 (10)	1 (10)	1 (10)	1 (10)	5 (12)
2.35	A	0 (0)	0 (0)	0 (0)	0 (0)	
	B	0 (0)	0 (0)	0 (0)	0 (0)	
	C	0 (0)	0 (0)	1 (10)	1 (10)	
	D	1 (10)	1 (10)	1 (10)	1 (10)	2 (5)
6.40	A	0 (0)	0 (0)	0 (0)	0 (0)	
	B	0 (0)	0 (0)	1 (10)	1 (10)	
	C	0 (0)	0 (0)	0 (0)	0 (0)	
	D	0 (0)	0 (0)	1 (10)	1 (10)	2 (5)

^aTreatment mortality is the cumulative number of dead in all four replicates divided by the total number of animals exposed (i.e., 40).

^bMean mortality is statistically greater than the mean of the control ($\alpha = 0.05$).

Table 2 cont.

Mortality of Water Flea, *Daphnia magna*, Exposed to Oxine Copper Under Flow-Through Test Conditions

Mean Measured Concentration (µg/L; ppb)	Rep	Cumulative Number Dead (% Mortality)				Treatment ^a
		Day 3	Day 7	Day 14	Day 21	
10.4	A	0 (0)	0 (0)	0 (0)	0 (0)	
	B	0 (0)	0 (0)	1 (10)	2 (20)	
	C	0 (0)	0 (0)	0 (0)	0 (0)	
	D	0 (0)	0 (0)	0 (0)	0 (0)	2 (5)
18.2	A	0 (0)	0 (0)	0 (0)	0 (0)	
	B	0 (0)	0 (0)	0 (0)	0 (0)	
	C	0 (0)	0 (0)	1 (10)	1 (10)	
	D	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
35.1	A	0 (0)	0 (0)	0 (0)	0 (0)	
	B	0 (0)	0 (0)	0 (0)	0 (0)	
	C	0 (0)	0 (0)	0 (0)	0 (0)	
	D	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
70.9	A	3 (30)	3 (30)	4 (40)	4 (40)	
	B	9 (90)	10 (100)	10 (100)	10 (100)	
	C	1 (10)	3 (30)	4 (40)	4 (40)	
	D	3 (30)	3 (30)	7 (70)	7 (70)	25 ^b (62)

^aTreatment mortality is the cumulative number of dead in all four replicates divided by the total number of animals exposed (i.e., 40).^bMean mortality is statistically greater than the mean of the pooled controls ($\alpha = 0.05$).

Oxine Copper toX DER MRID 428990-05

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Table 5. Lengths of Water Flea, Daphnia magna, after 21 Days of Exposure to Oxine Copper

Mean Measured Concentration ($\mu\text{g/L}$; ppb)		Helmet-Spine Length (mm)											
	Rep												
Control	A	4.9	4.7	4.8	5.0	4.9	5.2	4.9	4.6	5.0			
	B	4.7	5.0	4.8	4.9	4.2	4.4	4.9	5.0				
	C	5.0	5.0	4.7	5.0	5.0	4.9	4.8	5.0	4.6			
	D	4.9	4.9	4.7	4.8	4.4	4.8	4.3	4.9	4.9	5.0		
		Mean (\pm SD) = 4.82										(0.22)	
Sol. Control	A	4.2	4.5	4.3	4.4	4.3	4.9	4.1	4.5				
	B	4.5	4.8	4.5	4.6	4.4	4.8	4.8	4.4	4.5			
	C	4.5	4.6	4.4	4.5	4.5	4.5	4.4	4.7	4.5			
	D	4.8	4.5	4.4	4.1	4.7	4.4	4.5	4.3	4.8			
		Mean (\pm SD) = 4.50										(0.20)	
2.35	A	4.0	4.3	4.8	4.5	4.6	4.5	4.5	4.5	4.3	4.5		
	B	4.3	4.6	4.5	4.4	4.2	4.5	4.6	4.4	4.5	4.4		
	C	4.3	4.0	4.1	4.4	4.8	3.9	4.1	4.1	4.4			
	D	3.9	4.5	4.6	4.2	4.6	4.5	4.6	4.1	4.0			
		Mean (\pm SD) = 4.37										(0.24)	
6.40	A	4.4	4.6	4.7	4.1	4.4	4.6	3.9	4.1	4.5	4.6		
	B	4.5	4.3	4.5	4.5	3.9	4.5	4.0	4.5	4.3			
	C	4.5	4.5	4.7	4.8	4.5	4.3	4.4	4.3	4.5	4.2		
	D	4.5	4.4	4.9	4.3	4.9	4.3	4.6	4.4	4.9			
		Mean (\pm SD) = 4.44										(0.24)	

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Page 5 cont.

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Rep	Helmet-Spine Length											
10.4	A	4.6	4.9	4.2	4.4	4.6	4.5	4.3	4.4	4.2	4.1		
	B	4.4	4.4	4.3	4.7	4.3	4.2	4.0	4.5				
	C	4.1	4.5	4.1	4.6	4.5	4.4	4.3	4.1	4.3	4.5		
	D	4.4	4.3	4.5	4.6	4.0	4.0	4.4	4.0	4.6	4.6		
		Mean ($\pm\text{SD}$) = 4.36 (0.22)*											
18.2	A	4.5	4.9	3.9	4.9	4.5	4.1	4.0	4.5	4.5	4.6		
	B	4.1	4.0	4.5	4.2	4.1	4.3	4.4	4.9	4.2	4.5		
	C	4.3	3.9	4.3	4.6	4.5	4.2	4.0	4.1	4.6			
	D	4.4	4.6	4.3	4.1	4.5	4.1	4.4	4.4	4.5	4.0		
		Mean ($\pm\text{SD}$) = 4.34 (0.27)*											
35.1	A	4.9	4.6	4.4	4.3	4.5	4.8	4.4	4.7	4.7	4.5		
	B	4.4	4.8	4.1	4.2	4.4	4.6	4.4	4.6	4.4	4.6		
	C	4.0	4.5	4.5	4.5	4.6	4.4	4.3	4.6	4.6	4.3		
	D	4.7	4.6	4.8	4.9	4.7	4.3	4.3	4.5	4.5	4.5		
		Mean ($\pm\text{SD}$) = 4.51 (0.20)											
70.9	A	5.4	5.1	5.0	5.3	5.1	5.4						
	B												
	C	4.6	4.9	5.2	4.9	4.6	4.8						
	D	4.7	5.0	4.8									
		Mean ($\pm\text{SD}$) = 4.99 (0.26)											

* Statistical reduction in growth of water fleas (treatment mean) as compared to the solvent control ($\alpha = 0.05$).

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NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

MOSSLER OXINE COPPER DAPHNIA MAGNA 11-18-93

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
70.9	40	25	62.5	0
35.1	40	0	0	0
18.2	40	1	2.5	0
10.4	40	2	5	0
6.4	40	2	5	0
2.35	40	2	5	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT
CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE
UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 63.8739

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET
BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT
BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND
100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS G H GOODNESS OF FIT PROBABILITY
7 2.969613 9.802742 0
A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.49464
95 PERCENT CONFIDENCE LIMITS = -1.081008 AND 4.070287

LC50 = 114.9408
95 PERCENT CONFIDENCE LIMITS = 25.21168 AND +INFINITY

LC10 = 16.24691
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

daphnia reproduction

File: dap Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	sol cont	4	10.025	10.025	13.363
2	cont	4	16.700	16.700	13.363
3	2.35	4	9.425	9.425	9.525
4	6.4	4	9.475	9.475	9.525
5	10.4	4	9.200	9.200	9.525
6	18.2	4	10.000	10.000	9.525
7	35.1	4	7.550	7.550	7.550
8	70.9	4	3.825	3.825	3.825

daphnia reproduction

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
sol cont	13.363				
cont	13.363	3.456	*	1.71	k= 1, v=24
2.35	9.525	0.518		1.79	k= 2, v=24
6.4	9.525	0.518		1.82	k= 3, v=24
10.4	9.525	0.518		1.83	k= 4, v=24
18.2	9.525	0.518		1.84	k= 5, v=24
35.1	7.550	2.563	*	1.84	k= 6, v=24
70.9	3.825	6.420	*	1.85	k= 7, v=24

s 1.366

Note: df used for table values are approximate when v > 20.

daphnia survival

File: dap

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	sol cont	4	0.875	1.214	1.321
2	cont	4	0.900	1.254	1.321
3	2.35	4	0.950	1.331	1.321
4	6.4	4	0.950	1.331	1.321
5	10.4	4	0.950	1.336	1.321
6	18.2	4	0.975	1.371	1.321
7	35.1	4	1.000	1.412	1.321
8	70.9	4	0.375	0.628	0.628

daphnia survival

File: dap

Transform: ARC SINE(SQUARE ROOT(Y))

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
sol cont	1.321				
cont	1.321	0.996		1.71	k= 1, v=24
2.35	1.321	0.996		1.79	k= 2, v=24
6.4	1.321	0.996		1.82	k= 3, v=24
10.4	1.321	0.996		1.83	k= 4, v=24
18.2	1.321	0.996		1.84	k= 5, v=24
35.1	1.321	0.996		1.84	k= 6, v=24
70.9	0.628	5.424	*	1.85	k= 7, v=24

s = 0.153

Note: df used for table values are approximate when v > 20.

Analysis of Variance

File: dap

Date: 11-17-1993

FILTER: None

N's, means and standard deviations based on dependent variable: LENGTH

* Indicates statistics are collapsed over this factor

Factors: T R	N	Mean	S.D.
* *	264	4.4754	0.2714
1 *	35	4.5029	0.1963
2 *	36	4.8194	0.2202
3 *	38	4.3684	0.2372
4 *	38	4.4421	0.2456
5 *	38	4.3632	0.2186
6 *	39	4.3436	0.2673
7 *	40	4.5100	0.2023
* 1	67	4.5075	0.2857
* 2	64	4.4547	0.2500
* 3	66	4.4485	0.2747
* 4	67	4.4896	0.2748
1 1	8	4.4000	0.2449
1 2	9	4.5889	0.1691
1 3	9	4.5111	0.0928
1 4	9	4.5000	0.2345
2 1	9	4.8889	0.1764
2 2	8	4.7375	0.2925
2 3	9	4.8889	0.1537
2 4	10	4.7600	0.2319
3 1	10	4.4500	0.2121
3 2	10	4.4400	0.1265
3 3	9	4.2333	0.2739
3 4	9	4.3333	0.2828
4 1	10	4.3900	0.2685
4 2	9	4.3333	0.2345
4 3	10	4.4700	0.1829
4 4	9	4.5778	0.2587
5 1	10	4.4200	0.2394
5 2	8	4.3500	0.2070
5 3	10	4.3400	0.1897
5 4	10	4.3400	0.2547
6 1	10	4.4400	0.3438
6 2	10	4.3200	0.2658
6 3	9	4.2778	0.2539
6 4	10	4.3300	0.2003
7 1	10	4.5800	0.1932
7 2	10	4.4500	0.2068
7 3	10	4.4300	0.1889
7 4	10	4.5800	0.1989

~~~~~  
 Fmax for testing homogeneity of between subjects variances: 13.73  
 Number of variances= 28 df per variance= 8.  
 ~~~~~

~~~~~  
 Analysis of Variance

Dependent variable: LENGTH

| Source           | df  | SS (H)  | MSS    | F      | P      |
|------------------|-----|---------|--------|--------|--------|
| Between Subjects | 263 | 19.3699 |        |        |        |
| T (TRT)          | 6   | 5.9688  | 0.9948 | 19.417 | 0.0000 |
| R (Ref)          | 3   | 0.1577  | 0.0526 | 1.026  | 0.3785 |
| TR               | 13  | 1.1521  | 0.0886 | 1.249  | 0.2218 |

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Date: 11-17-1993

### Post-hoc tests for factor T (TRT)

- 1 = solvent control
- 2 = negative control
- 3 = 2.35  $\mu\text{g ai./l}$
- 4 = 6.4 " "
- 5 = 10.4 " "
- 6 = 18.2 " "
- 7 = 35.1 " "

| Comparison | Bon-<br>ferroni |
|------------|-----------------|
| 1 < 2      | 0.0000          |
| 1 > 3      |                 |
| 1 > 4      |                 |
| 1 > 5      |                 |
| 1 > 6      |                 |
| 1 < 7      |                 |
| 2 > 3      | 0.0000          |
| 2 > 4      | 0.0000          |
| 2 > 5      | 0.0000          |
| 2 > 6      | 0.0000          |
| 2 > 7      | 0.0000          |
| 3 < 4      |                 |
| 3 > 5      |                 |
| 3 > 6      |                 |
| 3 < 7      |                 |
| 4 > 5      |                 |
| 4 > 6      |                 |
| 4 < 7      |                 |
| 5 > 6      |                 |
| 5 < 7      |                 |
| 6 < 7      | 0.0266          |

| Comparison | Bonferroni |
|------------|------------|
| 1 > 2      |            |
| 1 > 3      |            |
| 1 > 4      |            |
| 2 > 3      |            |
| 2 < 4      |            |
| 3 < 4      |            |

Data listing  
Date: 11-19-1993  
FILTER: None

File: dap

| Obs. | TRT | REP | LENGTH |
|------|-----|-----|--------|
| 1    | 1   | 1   | 4.2    |
| 2    | 1   | 1   | 4.5    |
| 3    | 1   | 1   | 4.3    |
| 4    | 1   | 1   | 4.4    |
| 5    | 1   | 1   | 4.3    |
| 6    | 1   | 1   | 4.9    |
| 7    | 1   | 1   | 4.1    |
| 8    | 1   | 1   | 4.5    |
| 9    | 1   | 2   | 4.5    |
| 10   | 1   | 2   | 4.8    |
| 11   | 1   | 2   | 4.5    |
| 12   | 1   | 2   | 4.6    |
| 13   | 1   | 2   | 4.4    |
| 14   | 1   | 2   | 4.8    |
| 15   | 1   | 2   | 4.8    |
| 16   | 1   | 2   | 4.4    |
| 17   | 1   | 2   | 4.5    |
| 18   | 1   | 3   | 4.5    |
| 19   | 1   | 3   | 4.6    |
| 20   | 1   | 3   | 4.4    |
| 21   | 1   | 3   | 4.5    |
| 22   | 1   | 3   | 4.5    |
| 23   | 1   | 3   | 4.5    |
| 24   | 1   | 3   | 4.4    |
| 25   | 1   | 3   | 4.7    |
| 26   | 1   | 3   | 4.5    |
| 27   | 1   | 4   | 4.8    |
| 28   | 1   | 4   | 4.5    |
| 29   | 1   | 4   | 4.4    |
| 30   | 1   | 4   | 4.1    |
| 31   | 1   | 4   | 4.7    |
| 32   | 1   | 4   | 4.4    |
| 33   | 1   | 4   | 4.5    |
| 34   | 1   | 4   | 4.3    |
| 35   | 1   | 4   | 4.8    |
| 36   | 2   | 1   | 4.9    |
| 37   | 2   | 1   | 4.7    |
| 38   | 2   | 1   | 4.8    |
| 39   | 2   | 1   | 5.0    |
| 40   | 2   | 1   | 4.9    |
| 41   | 2   | 1   | 5.2    |
| 42   | 2   | 1   | 4.9    |
| 43   | 2   | 1   | 4.6    |
| 44   | 2   | 1   | 5.0    |
| 45   | 2   | 2   | 4.7    |
| 46   | 2   | 2   | 5.0    |
| 47   | 2   | 2   | 4.8    |
| 48   | 2   | 2   | 4.9    |
| 49   | 2   | 2   | 4.2    |
| 50   | 2   | 2   | 4.4    |

|     |   |   |     |
|-----|---|---|-----|
| 51  | 2 | 2 | 4.9 |
| 52  | 2 | 2 | 5.0 |
| 53  | 2 | 3 | 5.0 |
| 54  | 2 | 3 | 5.0 |
| 55  | 2 | 3 | 4.7 |
| 56  | 2 | 3 | 5.0 |
| 57  | 2 | 3 | 5.0 |
| 58  | 2 | 3 | 4.9 |
| 59  | 2 | 3 | 4.8 |
| 60  | 2 | 3 | 5.0 |
| 61  | 2 | 3 | 4.6 |
| 62  | 2 | 4 | 4.9 |
| 63  | 2 | 4 | 4.9 |
| 64  | 2 | 4 | 4.7 |
| 65  | 2 | 4 | 4.8 |
| 66  | 2 | 4 | 4.4 |
| 67  | 2 | 4 | 4.8 |
| 68  | 2 | 4 | 4.3 |
| 69  | 2 | 4 | 4.9 |
| 70  | 2 | 4 | 4.9 |
| 71  | 2 | 4 | 5.0 |
| 72  | 3 | 1 | 4.0 |
| 73  | 3 | 1 | 4.3 |
| 74  | 3 | 1 | 4.8 |
| 75  | 3 | 1 | 4.5 |
| 76  | 3 | 1 | 4.6 |
| 77  | 3 | 1 | 4.5 |
| 78  | 3 | 1 | 4.5 |
| 79  | 3 | 1 | 4.5 |
| 80  | 3 | 1 | 4.3 |
| 81  | 3 | 1 | 4.5 |
| 82  | 3 | 2 | 4.3 |
| 83  | 3 | 2 | 4.6 |
| 84  | 3 | 2 | 4.5 |
| 85  | 3 | 2 | 4.4 |
| 86  | 3 | 2 | 4.2 |
| 87  | 3 | 2 | 4.5 |
| 88  | 3 | 2 | 4.6 |
| 89  | 3 | 2 | 4.4 |
| 90  | 3 | 2 | 4.5 |
| 91  | 3 | 2 | 4.4 |
| 92  | 3 | 3 | 4.3 |
| 93  | 3 | 3 | 4.0 |
| 94  | 3 | 3 | 4.1 |
| 95  | 3 | 3 | 4.4 |
| 96  | 3 | 3 | 4.8 |
| 97  | 3 | 3 | 3.9 |
| 98  | 3 | 3 | 4.1 |
| 99  | 3 | 3 | 4.1 |
| 100 | 3 | 3 | 4.4 |
| 101 | 3 | 4 | 3.9 |
| 102 | 3 | 4 | 4.5 |
| 103 | 3 | 4 | 4.6 |
| 104 | 3 | 4 | 4.2 |

|     |   |   |     |
|-----|---|---|-----|
| 105 | 3 | 4 | 4.6 |
| 106 | 3 | 4 | 4.5 |
| 107 | 3 | 4 | 4.6 |
| 108 | 3 | 4 | 4.1 |
| 109 | 3 | 4 | 4.0 |
| 110 | 4 | 1 | 4.4 |
| 111 | 4 | 1 | 4.6 |
| 112 | 4 | 1 | 4.7 |
| 113 | 4 | 1 | 4.1 |
| 114 | 4 | 1 | 4.4 |
| 115 | 4 | 1 | 4.6 |
| 116 | 4 | 1 | 3.9 |
| 117 | 4 | 1 | 4.1 |
| 118 | 4 | 1 | 4.5 |
| 119 | 4 | 1 | 4.6 |
| 120 | 4 | 2 | 4.5 |
| 121 | 4 | 2 | 4.3 |
| 122 | 4 | 2 | 4.5 |
| 123 | 4 | 2 | 4.5 |
| 124 | 4 | 2 | 3.9 |
| 125 | 4 | 2 | 4.5 |
| 126 | 4 | 2 | 4.0 |
| 127 | 4 | 2 | 4.5 |
| 128 | 4 | 2 | 4.3 |
| 129 | 4 | 3 | 4.5 |
| 130 | 4 | 3 | 4.5 |
| 131 | 4 | 3 | 4.7 |
| 132 | 4 | 3 | 4.8 |
| 133 | 4 | 3 | 4.5 |
| 134 | 4 | 3 | 4.3 |
| 135 | 4 | 3 | 4.4 |
| 136 | 4 | 3 | 4.3 |
| 137 | 4 | 3 | 4.5 |
| 138 | 4 | 3 | 4.2 |
| 139 | 4 | 4 | 4.5 |
| 140 | 4 | 4 | 4.4 |
| 141 | 4 | 4 | 4.9 |
| 142 | 4 | 4 | 4.3 |
| 143 | 4 | 4 | 4.9 |
| 144 | 4 | 4 | 4.3 |
| 145 | 4 | 4 | 4.6 |
| 146 | 4 | 4 | 4.4 |
| 147 | 4 | 4 | 4.9 |
| 148 | 5 | 1 | 4.6 |
| 149 | 5 | 1 | 4.9 |
| 150 | 5 | 1 | 4.2 |
| 151 | 5 | 1 | 4.4 |
| 152 | 5 | 1 | 4.6 |
| 153 | 5 | 1 | 4.5 |
| 154 | 5 | 1 | 4.3 |
| 155 | 5 | 1 | 4.4 |
| 156 | 5 | 1 | 4.2 |
| 157 | 5 | 1 | 4.1 |
| 158 | 5 | 2 | 4.4 |

|     |   |   |     |
|-----|---|---|-----|
| 159 | 5 | 2 | 4.4 |
| 160 | 5 | 2 | 4.3 |
| 161 | 5 | 2 | 4.7 |
| 162 | 5 | 2 | 4.3 |
| 163 | 5 | 2 | 4.2 |
| 164 | 5 | 2 | 4.0 |
| 165 | 5 | 2 | 4.5 |
| 166 | 5 | 3 | 4.1 |
| 167 | 5 | 3 | 4.5 |
| 168 | 5 | 3 | 4.1 |
| 169 | 5 | 3 | 4.6 |
| 170 | 5 | 3 | 4.5 |
| 171 | 5 | 3 | 4.4 |
| 172 | 5 | 3 | 4.3 |
| 173 | 5 | 3 | 4.1 |
| 174 | 5 | 3 | 4.3 |
| 175 | 5 | 3 | 4.5 |
| 176 | 5 | 4 | 4.4 |
| 177 | 5 | 4 | 4.3 |
| 178 | 5 | 4 | 4.5 |
| 179 | 5 | 4 | 4.6 |
| 180 | 5 | 4 | 4.0 |
| 181 | 5 | 4 | 4.0 |
| 182 | 5 | 4 | 4.4 |
| 183 | 5 | 4 | 4.0 |
| 184 | 5 | 4 | 4.6 |
| 185 | 5 | 4 | 4.6 |
| 186 | 6 | 1 | 4.5 |
| 187 | 6 | 1 | 4.9 |
| 188 | 6 | 1 | 3.9 |
| 189 | 6 | 1 | 4.9 |
| 190 | 6 | 1 | 4.5 |
| 191 | 6 | 1 | 4.1 |
| 192 | 6 | 1 | 4.0 |
| 193 | 6 | 1 | 4.5 |
| 194 | 6 | 1 | 4.5 |
| 195 | 6 | 1 | 4.6 |
| 196 | 6 | 2 | 4.1 |
| 197 | 6 | 2 | 4.0 |
| 198 | 6 | 2 | 4.5 |
| 199 | 6 | 2 | 4.2 |
| 200 | 6 | 2 | 4.1 |
| 201 | 6 | 2 | 4.3 |
| 202 | 6 | 2 | 4.4 |
| 203 | 6 | 2 | 4.9 |
| 204 | 6 | 2 | 4.2 |
| 205 | 6 | 2 | 4.5 |
| 206 | 6 | 3 | 4.3 |
| 207 | 6 | 3 | 3.9 |
| 208 | 6 | 3 | 4.3 |
| 209 | 6 | 3 | 4.6 |
| 210 | 6 | 3 | 4.5 |
| 211 | 6 | 3 | 4.2 |
| 212 | 6 | 3 | 4.0 |

|     |   |   |     |
|-----|---|---|-----|
| 213 | 6 | 3 | 4.1 |
| 214 | 6 | 3 | 4.6 |
| 215 | 6 | 4 | 4.4 |
| 216 | 6 | 4 | 4.6 |
| 217 | 6 | 4 | 4.3 |
| 218 | 6 | 4 | 4.1 |
| 219 | 6 | 4 | 4.5 |
| 220 | 6 | 4 | 4.1 |
| 221 | 6 | 4 | 4.4 |
| 222 | 6 | 4 | 4.4 |
| 223 | 6 | 4 | 4.5 |
| 224 | 6 | 4 | 4.0 |
| 225 | 7 | 1 | 4.9 |
| 226 | 7 | 1 | 4.6 |
| 227 | 7 | 1 | 4.4 |
| 228 | 7 | 1 | 4.3 |
| 229 | 7 | 1 | 4.5 |
| 230 | 7 | 1 | 4.8 |
| 231 | 7 | 1 | 4.4 |
| 232 | 7 | 1 | 4.7 |
| 233 | 7 | 1 | 4.7 |
| 234 | 7 | 1 | 4.5 |
| 235 | 7 | 2 | 4.4 |
| 236 | 7 | 2 | 4.8 |
| 237 | 7 | 2 | 4.1 |
| 238 | 7 | 2 | 4.2 |
| 239 | 7 | 2 | 4.4 |
| 240 | 7 | 2 | 4.6 |
| 241 | 7 | 2 | 4.4 |
| 242 | 7 | 2 | 4.6 |
| 243 | 7 | 2 | 4.4 |
| 244 | 7 | 2 | 4.6 |
| 245 | 7 | 3 | 4.0 |
| 246 | 7 | 3 | 4.5 |
| 247 | 7 | 3 | 4.5 |
| 248 | 7 | 3 | 4.5 |
| 249 | 7 | 3 | 4.6 |
| 250 | 7 | 3 | 4.4 |
| 251 | 7 | 3 | 4.3 |
| 252 | 7 | 3 | 4.6 |
| 253 | 7 | 3 | 4.6 |
| 254 | 7 | 3 | 4.3 |
| 255 | 7 | 4 | 4.7 |
| 256 | 7 | 4 | 4.6 |
| 257 | 7 | 4 | 4.8 |
| 258 | 7 | 4 | 4.9 |
| 259 | 7 | 4 | 4.7 |
| 260 | 7 | 4 | 4.3 |
| 261 | 7 | 4 | 4.3 |
| 262 | 7 | 4 | 4.5 |
| 263 | 7 | 4 | 4.5 |
| 264 | 7 | 4 | 4.5 |